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1 **Partial overlap of fungal communities associated with nettle and poplar roots**
2 **when co-occurring at a trace metal contaminated site**

3
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20 **Summary**

21 Stinging nettle (*Urtica dioica* L.) raises growing interest in phytomanagement because
22 it commonly grows under poplar Short Rotation Coppices (SRC) set up at trace-metal (TM)
23 contaminated sites and provides high-quality herbaceous fibres. The mycobiome of this non-
24 mycorrhizal plant and its capacity to adapt to TM-contaminated environments remains
25 unknown. This study aimed at characterizing the mycobiome associated with nettle and poplar
26 roots co-occurring at a TM-contaminated site. Plant root barcoding using the fungi-specific
27 ITS1F-ITS2 primers and Illumina MiSeq technology revealed that nettle and poplar had distinct
28 root fungal communities. The nettle mycobiome was dominated by Pezizomycetes from known
29 endophytic taxa and from the supposedly saprotrophic genus *Kotlabaea* (which was the most
30 abundant). Several ectomycorrhizal fungi such as *Inocybe* (Agaricomycetes) and *Tuber*
31 (Pezizomycetes) species were associated with the poplar roots. Most of the Pezizomycetes taxa
32 were present in the highly TM-contaminated area whereas Agaricomycetes tended to be
33 reduced. Despite being a known non-mycorrhizal plant, nettle was associated with a significant
34 proportion of ectomycorrhizal OTU (9.7%), suggesting some connexions between the poplar
35 and the nettle root mycobiomes. Finally, our study raised the interest in reconsidering the fungal
36 networking beyond known mycorrhizal interactions.

37

38 **Keywords**

39 Ectomycorrhizal fungi ; Environmental metabarcoding ; Fungal network ; Metal-enriched
40 sediments ; Phytomanagement ; Root-associated mycobiome ; *Urtica dioica* L..

41

42 1. Introduction

43 Phytomanagement is a remediation technique based on plant species to restore and
44 revalorize polluted sites while producing biomass (Cundy et al., 2016; Evangelou et al., 2015).
45 Fast-growing trees such as poplars (*Populus sp.*) allow the production of a high amount of
46 valuable plant biomass for renewable energy (Kidd et al., 2015; Pandey et al., 2016). At the
47 same time, trees limit the dispersal and risks of soil contaminants, while promoting the
48 spontaneous biodiversity (Parraga-Aguado et al., 2014). Recent approaches on the optimisation
49 of phytomanagement systems pointed out the importance of assemblages between crops and
50 spontaneous species (Parraga-Aguado et al., 2013) to ensure a more efficient and gentle
51 remediation (Boisson et al., 2016). The spontaneous vegetation biodiversity is generally
52 restricted by the soil physico-chemical properties, limiting the panel of species able to develop
53 and compete with the tolerant species (Macnair, 1987). Much less is known on how this plant
54 biodiversity is shaping its root microbiota in this context.

55 The stinging nettle (*Urtica dioica* L.) colonizes a vast diversity of nitrophilic
56 environments and particularly anthropogenic areas (Balabanova et al., 2015; Shams et al., 2010;
57 Viktorova et al., 2016). It has thus been frequently observed as a companion species in a wide
58 range of nitrogen-rich environments such as Salicaceous plantations at both natural (Cronk et
59 al., 2016) and anthropized sites (Yung et al., 2019). Nettle has recently raised growing interest
60 in the domain of phytomanagement because it (i) constitutes a new resource of high-quality
61 herbaceous fibres for the manufacture of biomaterials (Bacci et al., 2009; Di Virgilio et al.,
62 2015; Jeannin et al., 2019, 2020), (ii) promotes the biodiversity of local entomofauna (James et
63 al., 2015; Perrin, 1975; Yung et al., 2019) and (iii) spontaneously grows in contaminated sites
64 (Murtic et al., 2019; Paukszto and Mirosławski, 2019; Viktorova et al., 2016).

65 The success of phytomanagement depends on the capacity of plants to colonize harsh
66 environments, which is partly related to their ability to interact with soil microorganisms, and

67 especially to establish symbiotic relationships with some of them. Among these
68 microorganisms, arbuscular mycorrhizal (AM) (Miransari, 2017), ectomycorrhizal (EM) (Gil-
69 Martínez et al., 2018) fungi and Dark Septate Endophytes (DSE) (Deng and Cao, 2017) are
70 major drivers of plant growth and response to abiotic stress and phytopathogens (Otero-Blanca
71 et al., 2018; Selosse et al., 2004). The use of fungal inoculum has been described as a
72 phytoremediation enhancer by improving uptake and accumulation of trace metals (TM) by
73 plants in phytoextraction application or by limiting the translocation of TM from roots to shoots
74 during phytostabilisation (Berthelot et al., 2016a; Khan, 2005; Ma et al., 2019; Pepper et al.,
75 2015). While the success of field inoculation is rather unpredictable (Gerhardt et al., 2017;
76 Selosse et al., 1998, 1999) due to various abiotic (*e.g.*, sensitivity to TM) and biotic parameters
77 (*e.g.*, competition with the local microbiome), the risks of failure may be mitigated by
78 considering indigenous strains, which may have developed a tolerance for TM (Wubs et al.,
79 2016). Characterisation of *in-situ* plant-associated microbiome appears as an essential
80 preliminary step for identifying rhizospheric microorganisms that may actively participate to
81 phytomanagement processes.

82 In the temperate climate zone, poplars are among the few cultivated trees that form
83 tripartite symbiotic associations with EM and AM fungi (Gehring et al., 2006; Teste et al.,
84 2020). As symbiotic fungi play crucial roles in soil fertility, colonisation, plant nutrition, metal
85 uptake, accumulation or restriction in plants (Smith and Read, 2010; van der Heijden et al.,
86 2015), poplar microbiome have been well studied in phytomanagement contexts (Durand et al.,
87 2017; Foulon et al., 2016b; Schmidt et al., 2018; Vitali et al., 2019; Zappelini et al., 2015).
88 Recent studies highlighted the positive effects of EM and AM inoculation on poplar cultivars
89 growing at a multi-contaminated site (Ciadamidaro et al., 2017; Phanthavongsa et al., 2017).
90 However, mycobiome associated with nettle have been barely studied in natural or
91 anthropogenic biota. A study combining *in-situ* and mesocosm approaches concluded that the

92 nettle rhizosphere was devoid of any mycorrhizal structures and revealed that the AM fungus
93 *Glomus mosseae* was not able to develop in the presence of nettle (Vierheilig et al., 1996). The
94 authors speculated that a rhizome protein called *Urtica dioica* agglutinin (UDA) with anti-
95 fungal properties (Broekaert et al., 1989) could be involved in AM avoidance. In addition, this
96 inhibitory effect could even affect the mycobiome of neighbouring plants (Fontenla et al.,
97 1999).

98 The present study aimed at characterizing, using Illumina Mi-seq sequencing, the
99 mycobiome associated with nettle and poplar roots co-occurring in an agrosystem set up on a
100 contaminated site, where two areas were clearly distinguished by their TM concentrations. We
101 hypothesised that poplar and nettle would harbour distinct root-associated fungal communities:
102 poplar mycobiome would be dominated by EM and AM fungi; at the opposite, nettle
103 communities would be composed of endophytic fungi (*i.e.* fungi colonizing root tissues
104 asymptotically (Wilson, 1995)) with a low fungal diversity as described above. We further
105 hypothesised that the level of TM contamination would alter the root-associated mycobiomes
106 by reducing root fungal diversity and by increasing the diversity and abundance of TM-tolerant
107 fungi, as mentioned elsewhere (Giller et al., 2009; Zappellini et al., 2015).

108

109 **2. Material and methods**

110 ***2.1. Site description***

111 The study site located at Fresnes-sur-Escaut (France, 50°25'47.9"N 3°35'07.8"E) is a
112 nettle-poplar agrosystem on a dredged sediment disposal site, contaminated with TM, that
113 operated between 1978 and 1989.

114 Approximately 200 000 m³ of silt and sand from the dredging of l'Escaut canal were
115 deposited, resulting in heterogeneous contamination with As, Cd, Cu, Ni, Pb and Zn within the
116 surface horizons (0-50 cm). The experimental site is divided into two areas differing by the
117 level of TM: the low trace-metal (LTM) and the high trace-metal (HTM) areas. The HTM area

118 has mean soil concentrations of Zn (2231 mg/kg), Pb (425 mg/kg), Cu (108 mg/kg) and Cd (63
119 mg/kg), on average 20-, 9-, 4- and 100-fold higher than the LTM area, respectively
120 (Phanthavongsa et al., 2017). A phytomanagement field trial was implemented in 2011 on this
121 site (covering both areas) with plots of poplar Skado (*P. trichocarpa* × *P. maximowiczii*) or
122 I214 (*P. deltoides* × *P. nigra*) as a short-rotation coppice (SRC; 2200 stems/ha). A detailed
123 description of the experimental design is provided in Ciadamidaro et al., (2017) and
124 Phanthavongsa et al., (2017). Five years after planting, the spontaneous herbaceous layer
125 evolved in the same way as that of another similar experiment set up in eastern France, with a
126 dominance of the stinging nettle under poplars with a coverage rate depending on the poplar
127 cultivar (Yung et al., 2019). In order to get rid of this variable and appreciate the contaminant
128 effect on the microbiomes associated with nettle and poplar roots, we selected only one plot for
129 each poplar cultivar (*i.e.* Skado or I214) for both the HTM and LTM areas, each of 504 m² (28m
130 x 18m), *i.e.* 4 plots in all. These plots are of sufficient size to allow for replicate collection.

131 ***2.2. Sample collection and preparation***

132 The sampling was conducted in November 2016, consisting of root samples from the
133 upper 20-cm layer of soil from 9 random poplars and 9 adjacent nettles for each of the four
134 studied plots. Comparisons between soil and poplar root mycobiomes were done and available
135 in our previous studies (Durand et al., 2017; Foulon et al., 2016b, 2016a). A total of three
136 pseudo-replicates of thin roots were sampled from each tree and mixed to obtain one composite
137 per poplar individual, whereas the entire root system of nettles was collected. For each plant,
138 the root samples were carefully sorted so as not to be mixed, the soil was carefully removed in
139 three successive baths in sterilized distilled-water, and the smallest roots were selected and
140 separated from larger roots by sampling them with a scalpel. The samples were freeze-dried
141 and stored at −20 °C before molecular analysis. Thus, we considered both the endophytic and
142 epiphytic fungi from the rhizoplane.

143 ***2.3. Molecular method***

144 The environmental DNA from the roots was extracted with the PowerSoil DNA
145 isolation Kit following the manufacturer's instructions (MoBio Laboratories, Inc., Carlsbad,
146 CA, USA). The PCR was performed using the specific fungal primers ITS1F (5'-
147 CTTGGTCATTTAGAGGAAGTAA-3') and ITS2 (5'-GCTGCGTTCTTCATCGATGC-3')
148 (Gardes and Bruns, 1993; White et al., 1990). We selected these primers to allow for
149 comparison with our previous dataset obtained on a similar plantation, at a different site
150 (Durand et al., 2017). These primers has also been found as one of the most competent in
151 uncovering the fungal diversity of plant root (Bainard et al., 2017; Huang et al., 2020). PCR
152 was performed in a reaction volume of 35 μ L using 0.5 μ M forward and reverse primer each,
153 200 μ M dNTPs, 1 μ L of Phire hot start II DNA polymerase (1 U/ μ l), 26.25 μ L of 5X Phire
154 reaction buffer (Thermo Fisher Scientific, Inc. USA), 10 ng template DNA and sterilized water
155 to reach a final volume of 35 μ l. The PCR conditions were 98 $^{\circ}$ C for 30 sec, followed by 25
156 cycles of 98 $^{\circ}$ C for 5 sec, 57.2 $^{\circ}$ C for 5 sec and 72 $^{\circ}$ C for 15 sec, followed by a final extension
157 step at 72 $^{\circ}$ C for 1 min. These primers targeted a short portion of the fungal ITS region, resulting
158 in amplicons of small size (~300 bp), appropriated for Illumina sequencing. The DNA quality
159 and quantity were assessed by agarose gel electrophoresis using the ImageLab software (Bio-
160 Rad, USA) and with a QubitTM dsDNA HS Assay Kit (Invitrogen, Carlsbad, CA, USA) using
161 the Qubit 4 fluorometer. The partial fungal ITS region (Wang et al., 2015) of 6 and 7 of nine
162 poplar and nettle samples (i.e. with the concentration of environmental DNA required) were
163 sequenced with the Illumina MiSeq platform (Microsynth AG, Switzerland).

164 **2.4. Bioinformatic analysis**

165 The sequence data were processed using Mothur (Schloss et al., 2009). First, all raw
166 read pairs were joined at the overlapping region to create consensus sequences. Then several
167 steps of filtering were performed, consisting in removing homopolymers, ambiguous sequences
168 and sequences with an inappropriate length (shorter than 220 bp and greater than 400 bp). The
169 18S RNA region amplified by the ITS1F primer (Toju et al., 2012) was removed by *in-silico*

170 PCR to target precisely the ITS1 region. After quality filtering, reads were then clustered into
171 OTUs using the swarm tools (Mahé et al., 2015) with a difference value of 1 and fastidious
172 option enable. The OTU clustering method has been repeatedly shown to be powerful for
173 recovering the richness and composition of the fungal community (Bell et al. 2014; Durand et
174 al., 2017; Foulon et al., 2016b; Huang et al. 2020; Nguyen et al., 2016). We removed singleton
175 OTUs to avoid technical artefacts and overestimation of the number of species (Tedersoo et al.,
176 2010). The taxonomic assignment of OTUs was performed using the UNITE (Kõljalg et al.,
177 2013) database by a naïve Bayesian approach (Wang et al., 2007). The presence of plant
178 sequences was verified using the BLAST tool of the GenBank database and removed. Each
179 fungal OTU was further assigned to functional or morphological groups of fungi using
180 FUNguild (Nguyen et al., 2016). For each assignment, three confidence rankings (*i.e.*,
181 “possible”: suspected, “probable”: fairly certain, “highly probable”: absolutely certain)
182 referring to previously peer reviewed data were given. We only considered the functional and
183 morphological assignation with at least a “probable” confidence ranking. Finally, the guilds
184 related to a similar function were grouped to obtain a total of six main guilds, based on the
185 literature.

186 **2.5. Data analysis**

187 Alpha diversity indices (OTU richness, Chao estimation, Shannon diversity index,
188 inverse of the Simpson diversity index) were calculated using MOTHUR. Samples were
189 rarefied at 33 000 sequences, corresponding to the smallest number of sequences detected in a
190 sample. No sample was excluded. To verify that sequencing depth allowed covering most of
191 the fungal diversity of our root samples, Good’s coverage was calculated as $C = [1 - (n / N)] \times$
192 100 (%), where “n” is the number of OTUs, and “N” is the number of sequences (Good, 1953).

193 Statistical analyses were performed with R software v.3.6.1 (R Development Core
194 Team, 2019). All the considered variables were tested for their homoscedasticity (Bartlett or
195 Levene tests) and normal distribution (Shapiro–Wilk test) and compared using analysis of

196 variance (ANOVA) followed by a Tukey HSD test. Where necessary, data were “log”
197 transformed to improve normality. Data that did not fit a normal distribution after
198 transformation were analysed with non-parametrical tests (Kruskal-Wallis test by ranks). First
199 we calculated and drew rarefaction curves for each experimental condition of nettle and poplar,
200 using “rarecurve” function available in the R Vegan package. The shape of the curve is a graphic
201 display of the relative estimated species accumulation rates. All diversity and richness indices
202 and the mean number of OTUs were then compared between all experimental conditions and
203 plants through ANOVA followed by a Tukey HSD test.

204 To summarize differences in fungal OTU composition between the experimental
205 conditions for both the I214 and Skado plots, we used two-dimensional non-metric Multi-
206 Dimensional Scaling (NMDS) plots based on Bray-Curtis dissimilarity matrix ($k = 2$) using the
207 metaMDS function of the R Vegan package. Permutational multivariate analyses of variance
208 (PERMANOVA) based on Bray-Curtis dissimilarity were applied to determine the potential
209 effects of the plant species (nettle vs poplar) and the level of contamination (LTM vs HTM) on
210 fungal communities for both I214 and Skado plots. To assess the influence of the studied factors
211 on the fungal classes, genera, EM fungal taxa and guilds, the experimental conditions were
212 compared using a Kruskal-Wallis test, followed by a post-hoc test with a Holm correction of
213 the p-value.

214 NMDS and PERMANOVA analyses were further performed on the EM fungal guilds.
215 For the whole fungal community and the six most abundant guilds taken individually, for nettle
216 and poplar, we tested the correlation and the overlapping between the OTU composition using
217 Mantel test (ade4 package) and by calculating the Morisita-Horn similarity index (SpadeR
218 package), respectively. The relative abundances of EM fungal OTUs were represented by a
219 heatmap, from the pheatmap R package. Similarly, a hierarchical cluster analysis of the relative

220 abundances of EM fungal OTUs from the various experimental conditions was represented by
 221 a dendrogram expressing the linkage between species and between the experimental conditions.

222

223 3. Results

224 3.1. Diversity of mycobiome associated with nettle and poplar roots

225

226 Illumina MiSeq sequencing of ITS1 amplicons obtained from 26 root samples of poplar
 227 and 25 root samples of nettle generated a total of 3,481,223 non-chimeric fungal sequences.
 228 The initial total number of sequences obtained per sample ranged from 33,615 to 140,409. The
 229 sample with the lowest number of sequences was selected for subsampling, revealing a total
 230 number of 1833 non-singleton OTUs for 1,683,000 fungal reads, and constituting our final
 231 dataset.

232 *Table 1: Results of the Illumina MiSeq sequencing of ITS1 amplicons, including richness and*
 233 *diversity indices of the mycobiomes associated to I214 and Skado poplar roots, or to nettle*
 234 *roots growing in the I214 or Skado plots, at low trace-metal (LTM) and the high trace-metal*
 235 *(HTM) areas. Mean values and standard deviations (mean ± SD) are provided for each*
 236 *experimental condition. The “total number of sequences” corresponds to datasets before*
 237 *subsampling at 33,000 sequences, while the other parameters are calculated after subsampling.*
 238 *Values designated with the same letters are not significantly different (Kruskal–Wallis test, P*
 239 *< 0.05).*

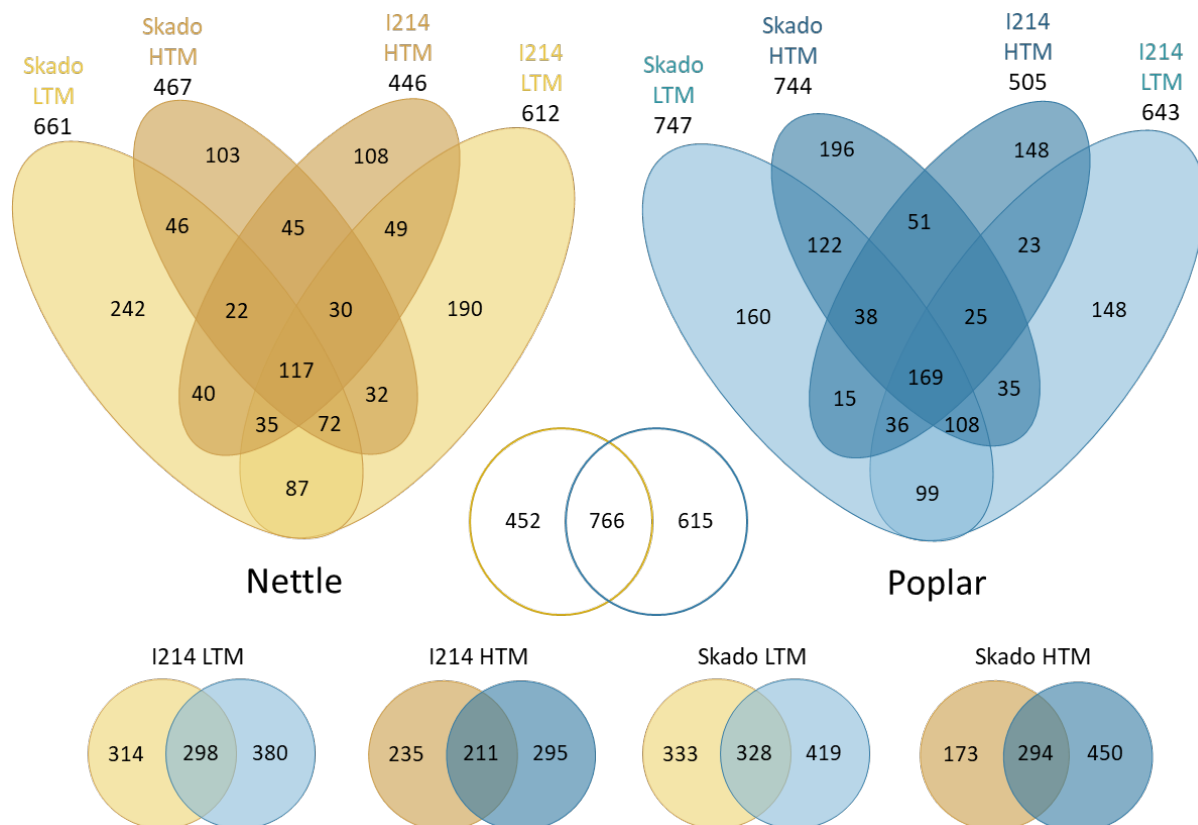
	NETTLE				POPLAR			
	LTM		HTM		LTM		HTM	
	I214	Skado	I214	Skado	I214	Skado	I214	Skado
Number of samples	6	6	6	7	6	7	6	7
Mean total number of sequences	52,264	73,778	68,434	70,185	69,479	63,116	55,243	61,067
Mean OTUs observed	175 ^{ab} (± 56)	168 ^{ab} (± 69)	122 ^b (± 23)	109 ^b (± 52)	178 ^{ab} (± 19)	196 ^a (± 30)	156 ^{ab} (± 38)	204 ^a (± 43)
Chao estimation	248 ^c (± 56)	280 ^{bc} (± 61)	221 ^c (± 25)	210 ^c (± 64)	362 ^{ab} (± 31)	410 ^a (± 74)	232 ^c (± 33)	358 ^{ab} (± 70)
Shannon index	2.3 ^{ab} (± 0.8)	2.7 ^a (± 1.0)	2.2 ^{ab} (± 0.6)	1.4 ^b (± 1.0)	2.4 ^{ab} (± 0.5)	2.7 ^a (± 0.5)	2.9 ^a (± 0.7)	2.9 ^a (± 0.5)
Inverse of Simpson diversity index	6.0 ^a (± 5.4)	8.9 ^a (± 6.1)	5.8 ^a (± 3.4)	3.1 ^a (± 2.8)	5.7 ^a (± 2.3)	7.7 ^a (± 5.3)	10.1 ^a (± 5.7)	8.9 ^a (± 6.1)

240

241 Rarefaction curves calculated for each experimental condition of nettle and poplar
 242 tended to reach an asymptotic profile, suggesting that the sequencing depth was sufficient to

243 cover the fungal diversity of our root samples. Additionally, the measured Good's coverage
244 values were greater than 99% for each condition and in every sample, suggesting a
245 representative sampling. Rarefaction curves for nettles clearly exhibited divergent trends in
246 LTM vs. HTM areas. For a same number of reads on the asymptote, the HTM plots reached a
247 number of OTUs reduced by approximately 30%, as compared to the LTM plots (Figure S1).
248 Concerning poplar, the curve for the I214-HTM plot was clearly lower than those corresponding
249 to the three other experimental conditions.

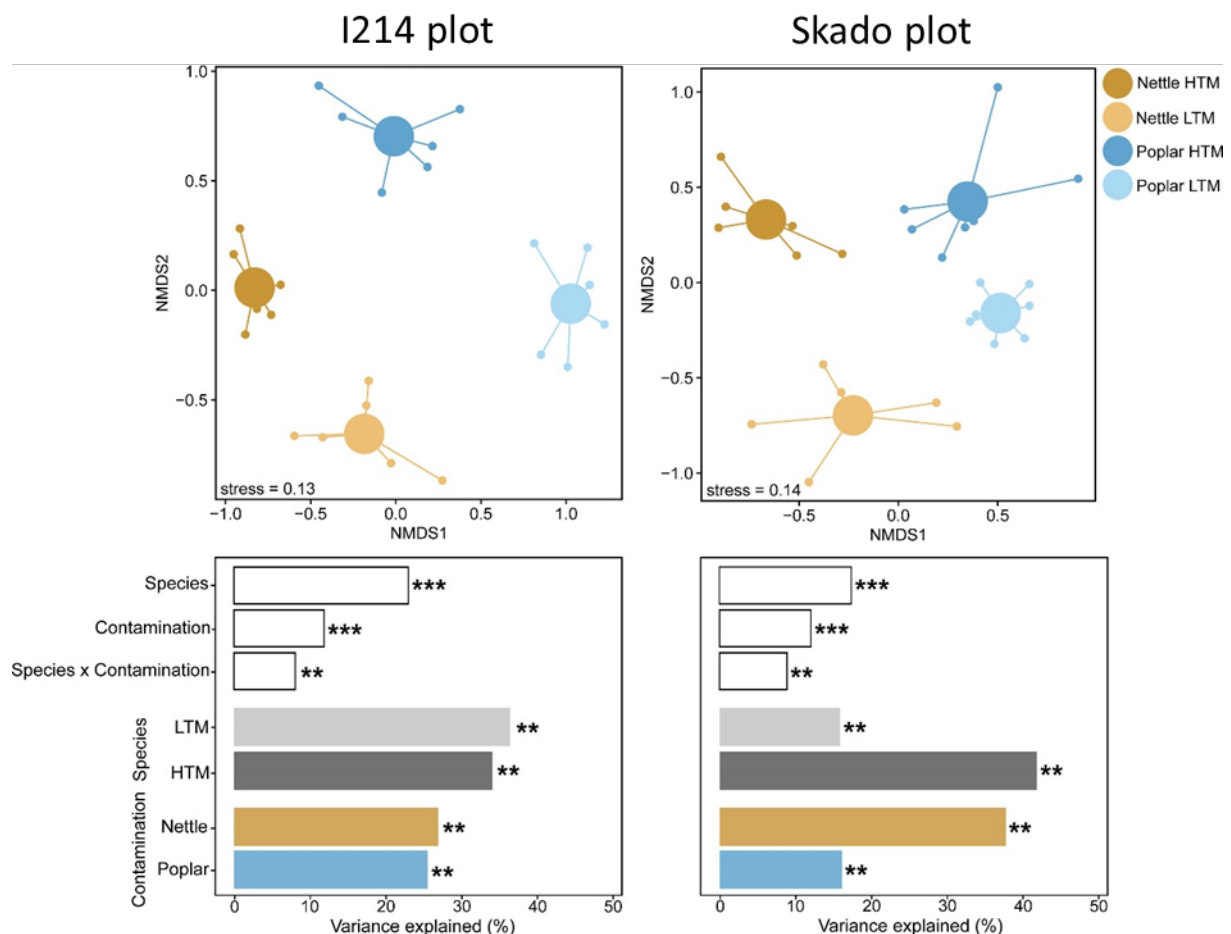
250 The total number of fungal OTUs was comparable for nettle (1218) and poplar (1321).
251 Among them, nettle and poplar shared 766 OTUs, representing 63% of the total number of
252 OTUs for nettle and 58% for poplar. The mean number of fungal OTUs associated with the
253 roots of nettle and poplar are detailed in Table 1 for each experimental condition. The highest
254 mean number of OTUs was obtained for Skado roots from the HTM area, whereas the lowest
255 was obtained for nettles within the same area, with a significant difference ($P < 0.05$). Only
256 7.1% and 7.5% of the total number of OTUs were shared between the four conditions for nettle
257 and poplar, respectively. Additionally, the percentage of OTUs specific to each condition
258 ranged between 8.5% and 19.9% for nettle and between 11.2% and 14.8% for poplar (Figure
259 1). For nettle, this number of specific OTUs was 2-fold higher in the LTM area (35.4%)
260 compared to the HTM area (17.3%), whereas it was comparable for poplar (23 vs 26%).
261 Depending on the experimental condition, nettle shared between 47.3% and 63.0% of their
262 OTUs with poplar, while poplar shared between 39.5% and 44.0% of their OTUs with nettle
263 (Figure 1).



264
 265 *Figure 1: Venn diagrams showing the overlap of the fungal communities according to the*
 266 *poplar cultivar plot (I214 and Skado) and the level of TM in soil (LTM and HTM), for nettle*
 267 *(left) and poplar (right). These diagrams present the number of total (above the ellipses),*
 268 *specific (within non-intersected ellipses) and shared OTUs (intersected ellipses). The five*
 269 *couples of circles provide the total number of OTUs for nettle (left) and poplar (right) and the*
 270 *shared number (intersection) for the whole dataset (center) and for each experimental*
 271 *conditions (bottom).*
 272

273 While the obtained mean numbers of fungal OTUs were comparable between nettle and
 274 poplar, the estimated number of fungal OTUs (using the Chao model) showed a higher species
 275 richness in poplar roots, except for the I214-HTM condition (Table 1). The species richness of
 276 the HTM area was lower than the LTM area for nettle, while this tendency was only noticeable
 277 for I214 poplars, as suggested by the rarefaction curves (Figure S1). For nettle, we collected an
 278 average of 65% and 54% of the estimated number of OTUs (Chao estimation) for the LTM and
 279 HTM areas, respectively, whereas we obtained 69% for HTM versus 48% for LTM for poplar.
 280 Overall, when considering the individual diversity estimators related to poplar or nettle, no
 281 apparent influence of the studied factors level of contamination was noticeable. However,
 282 NMDS analysis revealed a clear clustering of fungal communities according to the plant species

283 (nettle vs poplar) and the level of contamination (LTM vs HTM) for the I214 and Skado plots
 284 (Figure 2). Thus, while the fungal diversity did not seem to be significantly influenced by the
 285 experimental condition (Table 1), the fungal composition of nettle and poplar appeared
 286 contrasted and affected by the level of TM contamination and the plant species (Figure 2).
 287 NMDS and PERMANOVA indicated substantial differences in the composition of fungal
 288 communities between nettle and poplar (Figure 2), the plant species factor explaining 24% and
 289 17% of the variance in the fungal community for I214 and Skado plots, respectively ($P < 0.001$;
 290 Table S1). The level of TM had a significant effect on the fungal communities, both for nettle
 291 grown under I214 (28%) and Skado (37%) and for poplar, although to a lesser extent (26% for
 292 I214, and 16% for I214 Skado) (all $P < 0.01$; Figure 2, Table S1).



293

294 *Figure 2: Non-parametric multidimensional scaling (NMDS) plot of fungal communities*
 295 *associated with nettles and poplars grown at LTM and HTM areas, for I214 (left) and Skado*
 296 *(right) plots, using the Bray Curtis dissimilarity measure. Small and large circles represent the*
 297 *individual samples and the centroids of the different temperatures, respectively. Results from a*

298 *PERMANOVA* analysis for plant species (“species”) and level of TM (“contamination”)
299 factors and their interaction, and conducted separately by species (contamination effect) and
300 contamination (species effect) are provided under each NMDS, with the following legend: ***,
301 $P < 0.001$; **, $P < 0.01$; * $P < 0.05$.

302
303

304 **3.2. Taxonomic characterization of the mycobiome of nettle and poplar roots**

305 The fungal communities associated with roots of plants growing at the sediment disposal
306 site studied were dominated by the phylum *Ascomycota* in nettle roots (84.5% of total relative
307 abundance) whereas *Basidiomycota* (43.2%) and *Ascomycota* (52.3%) were more equally
308 represented in poplar roots (the *Ascomycota/Basidiomycota* ratio was significantly higher for
309 nettle than for poplar; $P < 0.05$).

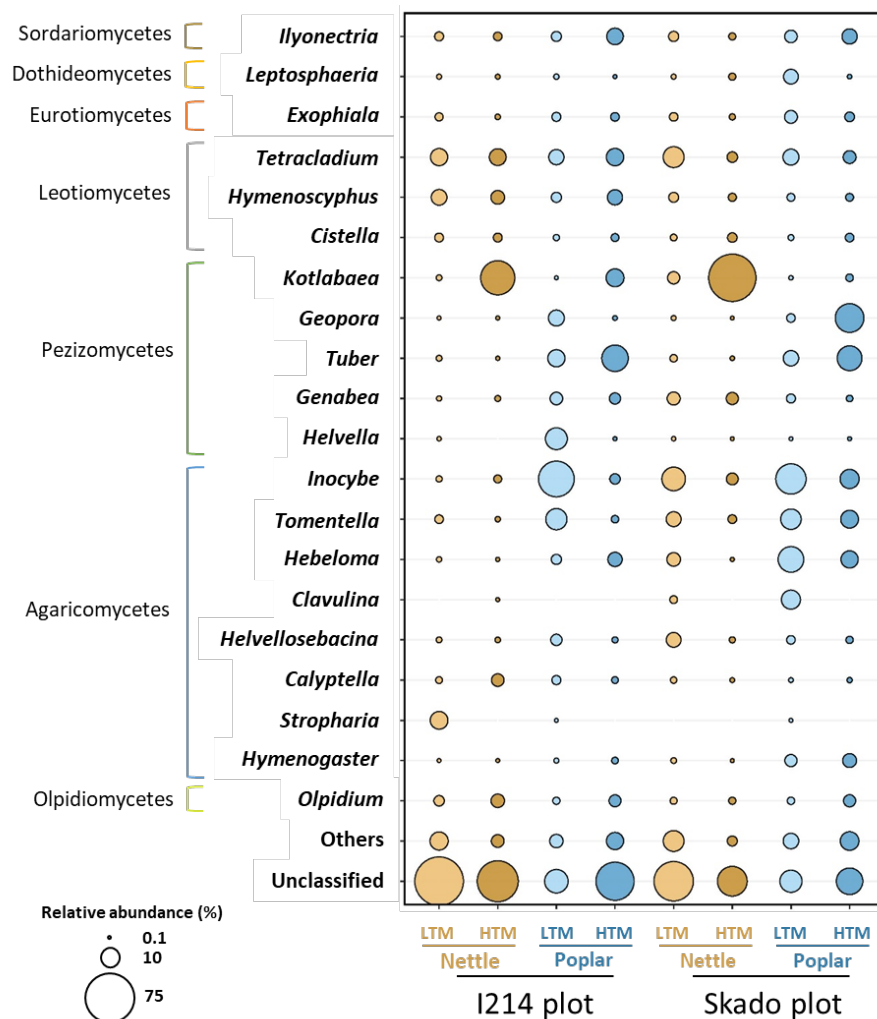
310 Among all samples, we detected 29 and 28 distinct fungal classes for nettle and poplar,
311 respectively. Among them, the most abundant classes were *Pezizomycetes*, *Agaricomycetes*,
312 *Leotiomycetes*, *Dothideomycetes*, *Sordariomycetes*, *Mortierellomycetes*, *Eurotiomycetes* and
313 *Olpidiomycetes* (total relative abundance of each $> 0.5\%$; Figure S2). For this study,
314 *Dothideomycetes* and *Leotiomycetes*, as abundant classes, characterized the mycobiome of
315 nettle roots. In contrast, poplar roots were characterized by *Agaricomycetes*, representing 20-
316 66% of all sequences, depending on the experimental condition (Figure S2). *Pezizomycetes*
317 were also well represented and similarly abundant in both plants.

318 For poplars, 79% of the fungal sequences were successfully assigned to a genus (73%
319 for I214 vs 85% for Skado), whereas only 54% were successfully assigned for nettle (Figure 3).
320 The genus *Kotlabaeva* was the most abundant genus associated in nettle roots (26.5% of the total
321 number of fungi associated with nettle), followed by three unknown genera belonging to
322 Helotiales (17.5%), Didymosphaeriaceae (8.7%) and Phaeosphaeriaceae (5.8%). The genera
323 *Tetracladium* (5.7%) and *Hymenoscyphus* (2.5%) were the most abundant nettle-associated
324 *Leotiomycetes* and were also present in poplar roots with comparable relative abundances
325 (Figure 3). For poplar, the most abundant genera were *Inocybe* (17.1% of the total number of

326 fungi associated with poplar), *Tuber* (10.8%), *Hebeloma* (7.3%), *Tomentella* (6.9%) and
327 *Geopora* (7.1%), the latter being almost absent in nettle roots (< 0.02%) (Figure 3). The genus
328 *Olpidium* (*Olpidiomycetes*), accounting for 1.3% and 1.4% of the fungal sequences for nettle
329 and poplar, respectively, was also well represented.

330 We detected significant differences in terms of fungal composition between the two
331 poplar cultivars. *Tomentella*, *Geopora*, *Hymenogaster*, *Hebeloma* and *Clavulina* were more
332 represented in roots of the Skado cultivar, while *Helvella* and *Kotlabaea* were mostly associated
333 with I214 cultivar ($P < 0.05$), but each of these trends were only significant for one of the two
334 considered areas (*i.e.*, LTM or HTM; $P < 0.05$; Figure 3, Table S2). Differences were also
335 noticed between mycobiomes of nettles grown in I214 plots (high density of nettle) and those
336 grown in Skado plots (low density of nettle). *Inocybe*, *Genabea*, *Helvellosebacina* and
337 *Hebeloma* were more abundant in roots of nettles grown in the Skado plot ($P < 0.05$), whereas
338 *Olpidium* and *Calyptella* were more abundant in the I214 plot ($P < 0.05$). These results were
339 significant for only one of the two considered areas (Figure 3, Table S2). Pezizomycetes were
340 significantly influenced by TM contamination : first, their relative abundance was significantly
341 higher at the HTM area for nettles, both the I214 (10% for LTM *vs.* 32% for HTM; $P < 0.05$)
342 and Skado (7% *vs.* 68%; $P < 0.05$) plots. Second, the same trend was observed on poplar but
343 only statistically supported for the Skado cultivar, (6% *vs.* 41%; $P < 0.05$; Figure S2). Among
344 Pezizomycetes, the genus *Kotlabaea* significantly increased in the HTM area (for I214 plots:
345 0.1% *vs.* 31.7%; for Skado plots: 2.5% *vs.* 65.1%; $P < 0.05$; Figure 3). Similarly, the genus
346 *Tuber* was more represented in the poplar roots collected from the HTM area (for the I214
347 cultivar: 6.2 % *vs.* 17.6%; for the Skado cultivar 4.6% *vs.* 15.2%; P), however, the high
348 between-sample variability did not allow to statistically support this. *Agaricomycetes* associated
349 with both poplar and nettle were influenced by TM as their abundance was significantly reduced
350 in the HTM areas ($P < 0.05$), except for nettle in the I214 plots (Figure S2). This reduction of

351 *Agaricomycetes* in the HTM area was mainly due to the decrease of the *Inocybe* genus, the most
 352 abundant of all *Agaricomycetes* for nettle (34%) and poplar (40%) (Figure 3). When considering
 353 nettle and poplar together, the relative abundance of *Inocybe* species decreased from LTM- to
 354 HTM area, namely from 31.9 to 1.5% for I214 plots and from 24.1 to 8.1% for Skado plots (P
 355 < 0.05 in each case; Figure 3). A similar but less pronounced trend was also noticeable for the
 356 genus *Tomentella* within the I214 plots.

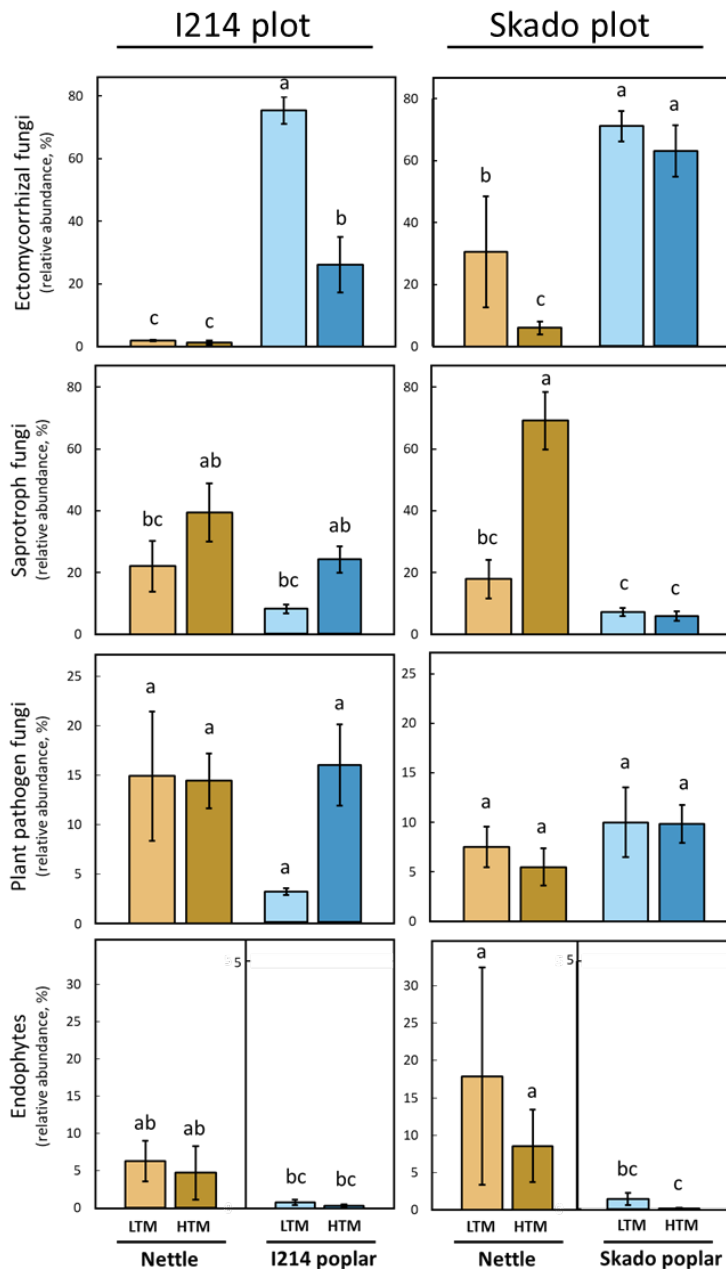


357
 358 *Figure 3: Relative abundance of the 20 most represented fungal genera (based on the*
 359 *taxonomic assignment of OTUs), belonging to 7 classes, according to the level of TM in soil*
 360 *(LTM and HTM) for nettle (orange bubbles) and poplar (blue bubbles) grown in the I214 or*
 361 *Skado plots. The classes with a relative abundance $< 0.5\%$ have been gathered in the “others”*
 362 *group. For each genus, the related results from Kruskal–Wallis pairwise or Tukey HSD tests*
 363 *are provided in the Table S2.*

3.3. Functional characterization of the mycobiome of nettle and poplar roots

366 Among the 1218 and 1321 fungal OTUs recorded in nettle and poplar roots (Figure 1),
367 70.4% and 83.1%, respectively, were successfully assigned to a functional guild with at least a
368 “probable” confidence ranking using FUNguild (Nguyen et al., 2016). This functional
369 assignment revealed a majority of saprotrophic fungi (from 18 to 69% depending on the
370 condition), followed by plant pathogens (6 - 15%) and EM fungi (1 - 30%) in the nettle roots
371 whereas EM fungi (26 - 75%), mainly represented by *Tomentella*, *Geopora*, *Hymenogaster*,
372 *Hebeloma* and *Clavulina* genera, were the most abundant guild in poplar roots (Figure 4). This
373 guild was significantly more abundant in roots of Skado poplar, compared to I214 poplar (63%
374 vs 26% for HTM area, $P < 0.05$; Figure 4). EM fungi were abundant on nettle roots and, for the
375 LTM area, even more on nettle grown with the Skado cultivar than on those grown with I214
376 (30% vs 2%; $P < 0.05$). Endophytes were poorly represented in the poplar roots in contrast to
377 nettle (0.1% vs. 9.2% of all sequences; $P < 0.05$; Figure 4). Twelve OTUs assigned to AM fungi
378 were found in nettle and poplar roots, accounting for less than 0.05% of all sequences (not
379 shown). Plant pathogenic fungi were equally represented in poplar and nettle roots, without any
380 influence of the studied factors (Figure 4).

381 Functional guild also revealed several differences between soil contamination levels.
382 Indeed, the relative abundance of nettle saprotrophs was higher in the HTM area (statistically
383 supported only for the Skado plots; $P < 0.05$) (Figure 4). This result was driven by *Kotlabaeta*,
384 the most abundant genus within this guild (Figure 3): an unidentified species from this genus,
385 accounting for 66% of all the saprotrophs associated with nettle, was indeed significantly more
386 present in the HTM areas ($P < 0.001$). EM fungi associated with poplar (particularly *Inocybe*
387 and *Tuber*) and nettle (particularly *Inocybe*) tended to decrease in the HTM area, which was
388 statistically supported for I214 plot in the case of poplar (75% vs 26%, $P < 0.05$) and for Skado
389 plot in the case of nettle (30% vs 6%; $P < 0.05$; Figure 4).



390

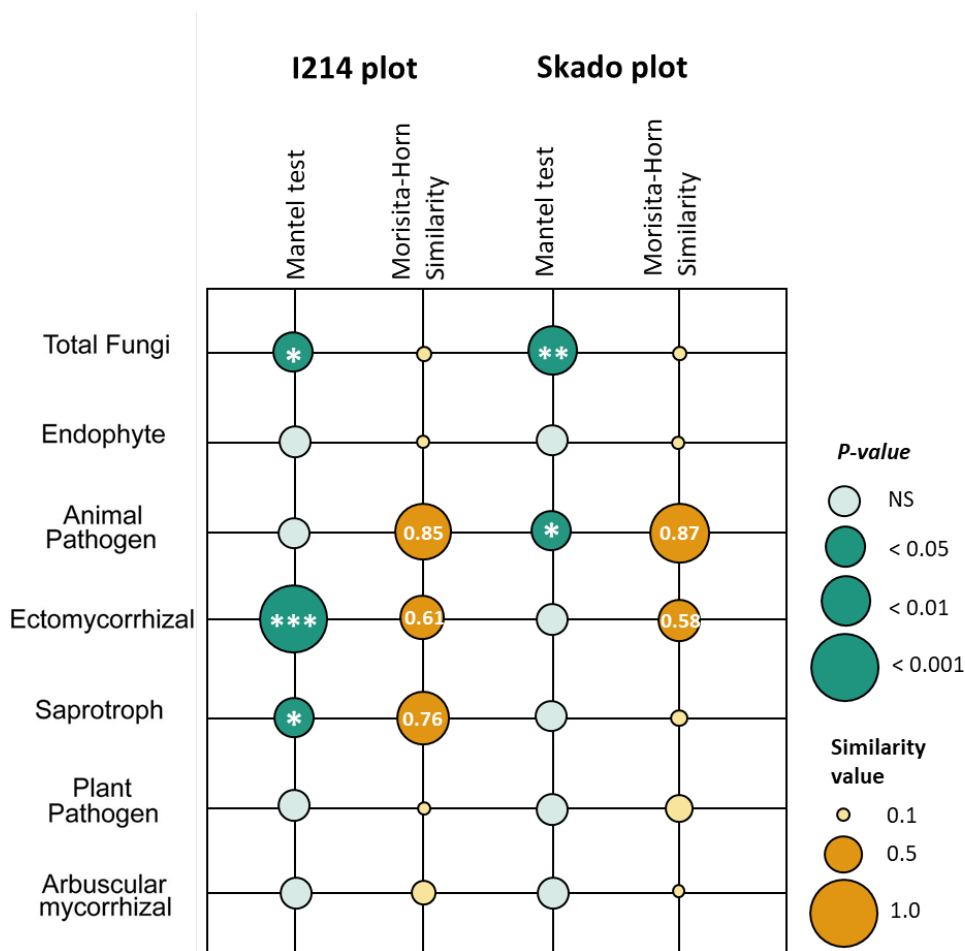
391 *Figure 4: Relative abundance of the four most abundant fungal guilds associated with nettles*
 392 *and poplars grown at the LTM or HTM area, for I214 (left) and Skado (right) plots. Data are*
 393 *the means ± SE (n = 6 or 7). For each class, bars designated with the same letter(s) are not*
 394 *significantly different (Kruskal–Wallis, P < 0.05).*

395

396 **3.4. Overlap of poplar and nettle microbial communities**

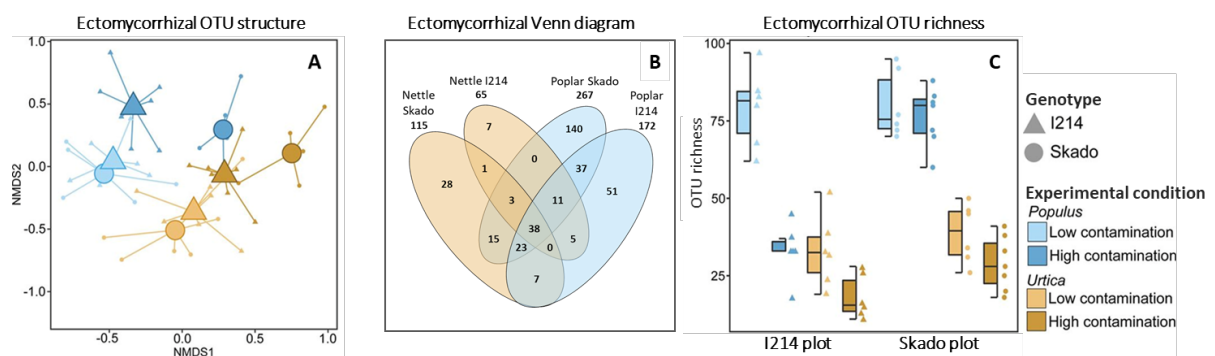
397 According to the Mantel test, nettle and poplar total fungal communities under I214 (P
 398 < 0.05) or Skado (P < 0.01) plots were significantly correlated (Figure 5), while the
 399 corresponding Morisita-Horn similarity index did not corroborate this result. However,
 400 similarity indexes and Mantel tests converged for nettle and poplar root-associated animal

401 pathogenic fungi, EM fungi and saprotrophs that were significantly correlated (Mantel test, $P <$
 402 0.05) and overlapped (Morisita-Horn index < 0.5 ; Figure 5). Despite the low relative abundance
 403 of EM fungi on nettle under I214 plots (Figure 4), nettle and poplar composition of EM fungi
 404 were strongly correlated ($P < 0.001$, Figure 5) for I214, while it was not the case for Skado
 405 plots. Interestingly, the saprotrophic guild of the two plant species were only correlated and
 406 overlapped at I214 plots, where the most abundant genera *Kotlabaea* was driving the trend.
 407 Consequently, these results suggested that nettle and poplar roots shared large part of their
 408 fungal communities.



409
 410 *Figure 5: Correlations (Mantel test) and similarity (Morisita-Horn index) between the OTUs*
 411 *composition of nettle and poplar, for the whole fungal community (“Total Fungi”) and the six*
 412 *most abundant guilds depending on the poplar cultivar plot. The size of the circles is*
 413 *proportional to the P-value for the Mantel test and to the index value for Morisita-Horn. Two*
 414 *communities with a Morisita-Horn index ≥ 0.5 are considered similar. P-value of the Mantel*
 415 *tests are represented with the following legend***, $P < 0.001$; **, $P < 0.01$; * $P < 0.05$.*

416 Although there was a significant relationship between EM fungi associated with the two
 417 plants, the NMDS analysis showed that EM fungi of nettle and poplar clustered for each host,
 418 but not together, suggesting host differentiation (Figure 6.A). EM fungi were significantly more
 419 represented (59.6% vs 9.7%; $P < 0.05$) and diverse (330 vs 146 OTUs; $P < 0.05$) in poplar than
 420 in nettle roots (Figure 4 and 6.C). Moreover, the ratios between nettle and poplar EM richnesses
 421 were similar for each condition (ranging from 0.64 to 0.83). A Venn diagram showed a higher
 422 species richness of EM fungi associated with poplar and particularly with the Skado cultivar
 423 (Figure 6.B), confirming poplar as the main host for EM fungi. As observed above for poplar,
 424 nettle EM fungi were more represented in terms of richness (115 vs 65 OTUs) and abundance
 425 (27.1 vs 1.7% of the total number of sequences) under the Skado plots as compared to the I214
 426 plots. Among the 138 OTUs associated with nettle, 102 OTUs were shared between nettle and
 427 poplar (Figure 6.B). Only 29 of the 115 EM OTUs (representing 1.6% of the total abundance
 428 of EM fungi) under the Skado plots and 8 of the 65 EM OTUs (11.7% of the total abundance)
 429 under the I214 plots were specific to nettle (Figure 6.B). These results suggested that most of
 430 the EM fungi associated with nettle were related to those hosted by poplar.



431
 432 *Figure 6: (A) Non-metric multidimensional scaling (NMDS) analysis of the EM fungal guild*
 433 *structure associated to poplar or nettle roots, (B) a Venn diagram based on EM OTUs and (C)*
 434 *the EM OTU richness, depending on the plant species (poplar or nettle), the poplar cultivar*
 435 *plot and the level of contamination (LTM and HTM).*
 436

437 According to a hierarchical cluster analysis of the relative abundances of EM fungal
 438 OTUs, LTM poplars were mostly colonized by the EM *Inocybe rimosa*, *Tomentella sp.*,

439 *Helvella elastica*, *Inocybe semifulva*, *Clavulina sp.* and *Hebeloma eburneum* (Figure S3). The
440 EM OTUs *Geopora cervina* and *Tuber rapaeodorum* were abundant in roots of poplar grown
441 at the HTM area (Figure S3). Most of these taxa were poorly represented in the nettle roots,
442 except for *Inocybe rimosa*, which was the most abundant EM fungus in both plants (Figure S3).
443 Indeed, this species accounted for 4% of nettle sequences (95% from the Skado plots) and
444 12.3% of poplar sequences (52% from the Skado plots). Additionally, this species was more
445 abundant in roots of nettle grown with the Skado cultivar ($P < 0.05$), and when associated with
446 poplar, it was sensitive to the soil contamination ($P < 0.05$) (Figure S3).

447

448 **4. Discussion**

449 **4.1. EM fungi dominated the poplar mycobiome**

450 In this study, the mycobiome associated with poplar roots were clearly dominated by
451 several EM fungal species, such as the Agaricomycetes *Inocybe rimosa*, *Tomentella sp.*, and
452 *Hebeloma spp.*, and the Pezizomycetes *Tuber rapeodorum* and *Geopora cervina*. We also
453 detected the presence of AM fungi belonging to the *Rhizophagus* and *Glomus* genera, although
454 with a rather low number of sequences ($< 0.001\%$), partially due to the choice that was made
455 to target the ITS1 region, which is not the most adapted region for the study of Glomeromycota
456 (Beeck et al., 2014). The recorded EM fungal species are known fungal symbionts of poplar
457 (Podila et al., 2009) that improve plant health by enhancing resistance to diverse stresses like
458 drought, salinity, heavy metals and pathogens, etc. (van der Heijden et al., 2015). Particularly,
459 they facilitate the adaptation of plants to TE stress, promoting host growth and
460 phytomanagement of TM-contaminated soils (Gil-Martínez et al., 2018).

461 In a previous study on a Hg-enriched sediment disposal site, the same experimental
462 design was set up in 2011. Durand et al. (2017) showed that EM fungi, and particularly the
463 *Tuber* and *Geopora* genera also dominated the roots of Skado poplar at this site. These taxa
464 seemed to be largely abundant in TM contaminated sites (Bonito et al., 2014; Guevara et al.,

465 2013; Lacerat-Didier et al., 2016). Moreover, these EM fungi were not only related to the
466 physico-chemical properties of these sites, but rather to the juvenil tree stage that promotes
467 some Pezizales (*Geopora spp.*, *Tuber spp.*) or Agaricales (*Inocybe spp.*) (Foulon et al., 2016b;
468 Hrynkiewicz et al., 2010; Lacerat-Didier et al., 2016). *Hebeloma* or *Inocybe* species, which
469 were well represented at the studied site, are “early stage” fungi capable of colonizing roots of
470 trees established in virgin or disturbed habitats (Smith and Read, 2010). In our dataset, only 7%
471 of the variability were explained by the cultivar factor when performing a PERMANOVA on
472 the poplar dataset (data not shown), indicating that the mycobiomes of Skado and I214 roots
473 were comparable, consistently our previous results (Foulon et al., 2016a): in our previous work,
474 the mycobiome of eight poplar cultivars grown at a TM-contaminated site, exhibited slight
475 differences, especially between I214 and Skado roots. Together with the present study, the only
476 discrepancy between the two communities concerned the *Laccaria* genus, only recorded under
477 the I214 cultivar in small abundance. This similarity between the two studies might be linked
478 to the similar TM composition of the two sites and the similar age of the poplar plantation (7-
479 years in Foulon *et al.*, (2016a) vs 6-years in the present study).

480 ***4.2. Saprotroph and endophytic fungi dominated the nettle root mycobiome***

481 The overall fungal composition of nettle roots differed from that of poplar, despite their
482 co-occurrence. Most of the dominant species associated with nettles were only assigned to the
483 family or order level, reflecting a lack of information concerning the nettle mycobiome.
484 Dominant species on nettle roots were mostly endophytic or pathogenic fungi, such as
485 *Tetracladium sp.*, *Olpidium spp.*, or saprotroph belonging to the Helotiales and Pleosporales
486 orders as previously observed for the non-mycorrhizal plant *Arabis alpina* (Almario et al.,
487 2017). The most abundant OTU was assigned to the *Kotlabaeya* genus, accounting for more than
488 26% of all sequences. This species belongs to the Pyronemataceae, one of the largest family of
489 Pezizales traditionally considered saprotrophic, although the trophic strategies of most species
490 remain undocumented (Perry et al., 2007). Indeed, an increasing number of them are being

491 identified as EM (Tedersoo et al., 2010). We classified *Kotlabaea* spp. as saprotrophic based
492 on (Tedersoo et al., 2014), but uncertainties remained. Overall, knowledge about taxonomy and
493 functional role of these root-associated fungi of nettle needs to be enriched by fungal isolation
494 and characterisation experiments, which has not yet been performed to our knowledge.

495 ***4.3. The degree of contamination differently affected the mycobiomes of poplar and*** 496 ***nettle***

497 According to a previous pedogeochemical characterization of the soils from the two
498 areas, Zn and Cd concentrations were the most contrasted parameters between the two areas
499 (Phanthavongsa et al., 2017). Other soil parameters were rather comparable, suggesting that the
500 level of TM is one of the main factor influencing the soil fungal communities.

501 A slight reduction of fungal diversity was noticed for plants grown at the most
502 contaminated area (HTM), which is commonly observed (Bååth, 1989; Giller et al., 2009).
503 Indeed, while certain fungal microorganisms can tolerate large concentrations of non-essential
504 heavy metals (*e.g.* Al, As, Cd, Hg, Pb), plant mycobiome is generally affected (Gadd, 2010).
505 Other studies showed that TM tended to reduce the amount of soil microbial biomass as well
506 as the species richness, changing microbial structure in favor of tolerant taxa and modifying the
507 soil functioning (Giller et al., 1998, 2009).

508 Some mycorrhizal fungi isolated from contaminated soils develop adaptations to
509 tolerate metals (Colpaert et al., 2011; Gonçalves et al., 2009), which make them particularly
510 suitable for phytomanagement applications. Indeed, plants inoculated with metal-tolerant EM
511 fungi (Adriaensen et al., 2005; Redon et al., 2009) or DSE (Likar and Regvar, 2013) showed
512 enhanced tolerance when compared to plants inoculated with non-tolerant strains. In our
513 observations, Agaricomycetes, and particularly the *Inocybe* and *Tomentella* genera, when
514 associated with nettle or poplar, were the most impacted by contamination. Although
515 significantly reduced, these two EM fungi were still well represented at the heavily
516 contaminated area on poplar roots. Additionally, the *Inocybe* genus has already been isolated

517 (Repáč, 1996), and successfully tested as an inoculum strain on coniferous trees (Flores-
518 Rentería et al., 2018), which supports its interest in bioremediation applications. Their
519 usefulness and functional importance in nettle plantation deserves further analyses (see below).

520 In our study, members of the Pezizomycetes seemed to be the most tolerant fungi as the
521 *Kotlabaea* genus associated with nettle and the *Tuber* genus, associated with poplars, were more
522 represented in the area with the highest concentration of Cd, Zn, Cu and Pb. This increase of
523 Perizomycetes related to soil contamination has already been detected in hydrocarbon-
524 contaminated soils (Bell et al., 2014; Tardif et al., 2016). Indeed, an untested possibility is that
525 hydrocarbons are also present at our site because canals dredged sediments generally contain
526 various contaminants (Besser et al., 1996; King et al., 2006).

527 ***4.4. A significant fraction of EM fungi were shared by poplar and nettle roots***

528 Despite the noticeable differences mentioned above in terms of fungal composition
529 between nettle and poplar, our results suggested that their respective mycobiome could be
530 influenced by the other when co-occurring. More specifically, fungal taxa associated with
531 abundant plants seemed more likely to colonize neighbouring plants than if the later were alone.
532 Indeed, taxa characteristics of the nettle mycobiome (e.g. *Helvella*, *Kotlabaea*) were more
533 represented in the I214 plot where nettle covered up to 60% of the herbaceous layer, compared
534 to the Skado plot where nettle and other plants were rare. Similarly, an influence of Skado
535 poplar on the mycobiome of neighbouring nettles was possibly detected, since a significant part
536 (9.7%) of EM OTUs, which were characteristic of poplars mycobiomes, contributed to the
537 mycobiome of nettle roots, which might be surprising as this plant is a recognised non-
538 mycorrhizal plant (Vierheilig et al., 1996). Nettle roots were carefully washed and sorted,
539 making contaminations by the adjacent soil or poplar roots unlikely. Yet their presence on the
540 rhizoplane or as true endophytes (*i.e.* with root penetration) deserves further investigations.

541 Indeed, the ability of some EM fungi to colonize AM (and more generally non-EM)
542 plants has already been described for several clades based on molecular detection and direct

543 observations, mainly for Sebacinaceae (Selosse et al., 2009; Weiß et al., 2011) and *Tuber* spp.
544 (Gryndler et al., 2014; Schneider-Maunoury et al., 2018, 2020). In addition, some suspicion
545 exists, based on molecular detection only, for Thelephoraceae and Inocybaceae (Schneider-
546 Maunoury et al., 2018, 2020), Pyronemataceae (Hansen et al., 2013) and Helotiales (Wang et
547 al., 2006). Such a dual, EM and endophytic interaction has been viewed as a persistence of the
548 evolutionary past of EM fungi, if they evolved from endophytic species: in the so-called
549 ‘waiting room hypothesis’, endophytism is considered as a niche from which the tighter and
550 more elaborate mycorrhizal symbiosis can evolve (Schneider-Maunoury et al., 2018; Selosse et
551 al., 2009; van der Heijden et al., 2015). It was recently commentated that many fungi have more
552 complex niches (*e.g.*, dual niches) than what is commonly agreed, which could be explained by
553 such evolutionary trajectories (Selosse et al., 2018, Thoen et al., 2020).

554 Recently, Taschen et al. (2020) demonstrated that *Tuber melanosporum* growth gains
555 benefit from the non-EM plants it colonizes, while it has a negative effect on them.
556 Additionally, the EM trees colonized by this fungus as ectomycorrhizal partners gain, at least
557 in terms of nitrogen content, from the interaction between truffles and non-EM plants. The
558 possibility of an overlooked interaction between EM- and AM- or non-mycorrhizal plants
559 growing close to host trees is further supported by the fact that connection and the exchange of
560 carbon between plant sharing the same belowground ectomycorrhizal network were
561 demonstrated for trees (Klein et al., 2016; Selosse et al., 2006), even when one of the plant was
562 herbaceous (Selosse et al., 2009). The functional implications for plants of an extensive sharing
563 of non-mycorrhizal fungi remained unclear and highly dependant on the plant-fungus
564 association. In case they are beneficial, and this happens even for non-mycorrhizal endophytic
565 fungi such as dark septate endophytes in non-mycorrhizal plants (Liu et al., 2017; Yung et al.,
566 2021), there may be mutual reinforcement of the two plant species by way of coordinated
567 responses to stresses, nutritional sharing, etc. Yet, the benefit can be asymmetric, or even

568 relevant for one plant species only. Moreover, an intriguing possibility is pathogen spillover
569 (Mordecai, 2011), *i.e.* one plant promotes the spread of pathogens that harm competitors more
570 strongly than itself. In this context, further investigations are now necessary to conclude about
571 the exact nature and functionality of this poplar-nettle EM sharing and its consequence for this
572 agroforestry system.

573 *Inocybe rimosa*, the most abundant EM fungal species found on nettle roots, accounting
574 for approximately 4% of the total fungal sequences, is a major candidate for such studies. The
575 presence of *I. rimosa* is reported in TM-polluted soils (Huang et al., 2012; Krpata et al., 2009)
576 and, although its ability to colonize roots of non-EM plant has not been previously investigated,
577 Inocybaceae were already detected in some AM plants (Schneider-Maunoury et al., 2018). Any
578 facilitation mechanism through EM fungi could contribute to the dominance of nettles within
579 Salicaceous SRC in natural (Cronk et al., 2016) and contaminated sites (Yung et al., 2019).
580 However, further research about the mycobiome of nettle roots and particularly in the case of
581 co-cropping at phytomanagement sites, especially to assess morphological (*e.g.* by fluorescent
582 *in-situ* hybridization; Schneider-Maunoury *et al.*, 2020) and functional evidence of interactions
583 with EM fungi, is now awaited. *I. rimosa* is present in the CBS Filamentous fungi and Yeast
584 Collection as CBS 210.55. It will be used in further experiments, for poplar x nettle experiments
585 to test its growth between the two plants and assess its positive or negative or no influence on
586 nettle.

587 As a conclusion, we taxonomically and functionally characterized the fungal
588 microbiomes of a spontaneous species (*i.e.*, *Urtica dioica* L.) co-occurring with cultivated crops
589 (*i.e.*, *Populus* spp.) at a phytomanaged area contaminated with TM. Our results suggested that
590 nettle and poplar had distinct root-associated fungal communities, albeit sharing many common
591 ectomycorrhizal fungi and particularly the genus *Inocybe*, which is surprising given the non-
592 mycorrhizal status of nettle. These results suggest to reconsider the ecological niches of fungi.

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602

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